Volume 322, number 2, 139–142 FEBS 12406 May 1993

Three-dimensional model of NAD$^+$-dependent 15-hydroxyprostaglandin dehydrogenase and relationships to the NADP$^+$-dependent enzyme (carbonyl reductase)

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Received 11 March 1993; revised version received 19 March 1993

Modelling the amino acid sequence of NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenase into the three-dimensional structure of 3α/20β-hydroxysteroid dehydrogenase shows that these two enzymes, as well as the NADP$^+$-linked prostaglandin dehydrogenase (identical to carbonyl reductase) have similar conformations, in spite of very limited sequence identity (23–28%). Conservation of tertiary structures is greatest over the first two thirds of the polypeptide chains, where the typical NAD$^+$ binding fold is retained, including the five first β-strands, with only two short deletions or insertions up to residue 147. The remaining thirds of each of the prostaglandin dehydrogenases have significantly different architecture, including insertions that may contribute to enzyme specificity, and, except for an additional helix (αG), are difficult to model. Active site relationships can be evaluated and subunit interactions predicted, suggesting that the αE + αF two-helix surface constitutes the major subunit interacting area, forming a dimeric unit in the oligomeric enzymes.

Computer graphics; Prostaglandin dehydrogenase; Short-chain dehydrogenases; Enzyme family; Insertion/deletion; Subunit interactions

1. INTRODUCTION

Two different cytosolic types of human prostaglandin dehydrogenase have been characterized, a monomeric NADP$^+$-linked enzyme identical to carbonyl reductase [1–4], and a dimeric NAD$^+$-linked enzyme [5]. Both belong to the short-chain dehydrogenase family, but they are highly divergent, exhibiting identical residues at the 20% level [1,2,6]. The tertiary structures of other members of this family, a tetrameric steroid dehydrogenase and a dimeric dihydropteridine reductase [7,8], have recently been crystallographically determined, and a model for NADP$^+$-linked prostaglandin dehydrogenase has been based upon these studies [1]. Although the model has a closely related conformation, it has several features not present in the observed structures, including elements of secondary structure, and a partly shifted architecture. However, all members of the short-chain dehydrogenase family are peculiar in having a single domain, with more (and a few longer) strands in the β-pleated sheet [1,7,8] than those found in corresponding parts of the medium-chain dehydrogenases [9].

Because of the low degree of homology, the conformation of the other prostaglandin dehydrogenase is also of interest, the NAD$^+$-linked enzyme from human placenta. In the present work, we compare by computer graphics the NAD$^+$-linked enzyme with the crystallographically analyzed steroid dehydrogenase [7], and relate the two prostaglandin dehydrogenases to each other.

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2. MATERIALS AND METHODS

2.1. *Sequence alignment*

The sequences of human placental NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenase [5] and 3α/20β-hydroxysteroid dehydrogenase from *Streptomyces hydrogenans* [10] were aligned using the program ALIGN [11] to maximize sequence identity between the two proteins. The alignment was then adjusted manually in order to conserve secondary structure. This was performed by shifting, where possible, computer-generated insertions and deletions from inside elements of secondary structure into the closest loop regions, if they had not already originally been introduced at such places.

2.2. *Model building*

The model of NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenase was built on an Evans and Sutherland PS 390 computer graphics system with the FRODO program [13,14] based on the coordinates of 3α/20β-hydroxysteroid dehydrogenase [7]. The amino acid sequence of 15-hydroxyprostaglandin dehydrogenase was transferred into the model by replacement and refinement of amino acids in groups of five residues. The torsion angles of the side-chains were adjusted to avoid unfavourable contacts with other side-chains. The model was energy minimized using the program XPLOR [15] that refined the positional parameters of the dimeric atoms. The energy minimization process converged after 200 cycles of refinement. The van der Waals and Electric energies were −2,382 kcal/mol and −7,585 kcal/mol, respectively. The bond distance, bond angle, and dihedral angle energies were 200 kcal/mol, 743 kcal/mol, and 533 kcal/mol, respectively. The two-fold rotational symmetry restraining energy between the subunits was 2 kcal/mol.

Two short insertions in the prostaglandin dehydrogenase relative to the steroid dehydrogenase had to be modelled. An insertion of five residues at the end of αC could be accommodated by extension of the α-helix by one residue, and modelling of the additional four residues into the loop connecting αC with βC. The other insertion, two residues at the end of αF, was modelled as an extension of the loop between αF and βF. A deletion of five residues at the end of αE was modelled by elimination of these residues from the end of the helix, leaving αE 1.5 turns shorter, but with similar end connections. Two segments of 15-hydroxyprostaglandin dehydrogenase, Ile^{190}–Pro^{222} and Lys^{249}–Glu^{266}, are not included in the model, because of too extensive differences in those parts.

3. RESULTS AND DISCUSSION

3.1. *Alignment*

The alignment now obtained between the NAD^+-linked 15-hydroxyprostaglandin dehydrogenase and corresponding parts of 3α/20β-hydroxysteroid dehydrogenase is shown in Fig. 1. Overall, the residue identity is 26% (excluding gaps); considering only the region modelled, the identity is 28%. The alignment is similar to that previously based on the primary structures [6] and similarities with the $NADP^+$-linked enzyme [1], except that gaps and insertions are now placed outside elements of secondary structure. Residues that are conserved in most short-chain dehydrogenases are conserved also in this alignment.

Alignment of the first 189 residues (through βF) presented no special problems. However, alignment beyond βF is difficult because there are few conserved residues in the remaining parts. The next element of secondary structure in 3α/20β-hydroxysteroid dehydrogenase, the helix αG (Met^{189}–Tyr^{202}), can be aligned with either Ile^{190}–Tyr^{203} of the NAD^+-linked prostaglandin dehydrogenase or shifted toward the C-terminus of the prostaglandin dehydrogenase. We chose the latter alternative, introducing a loop between βF and

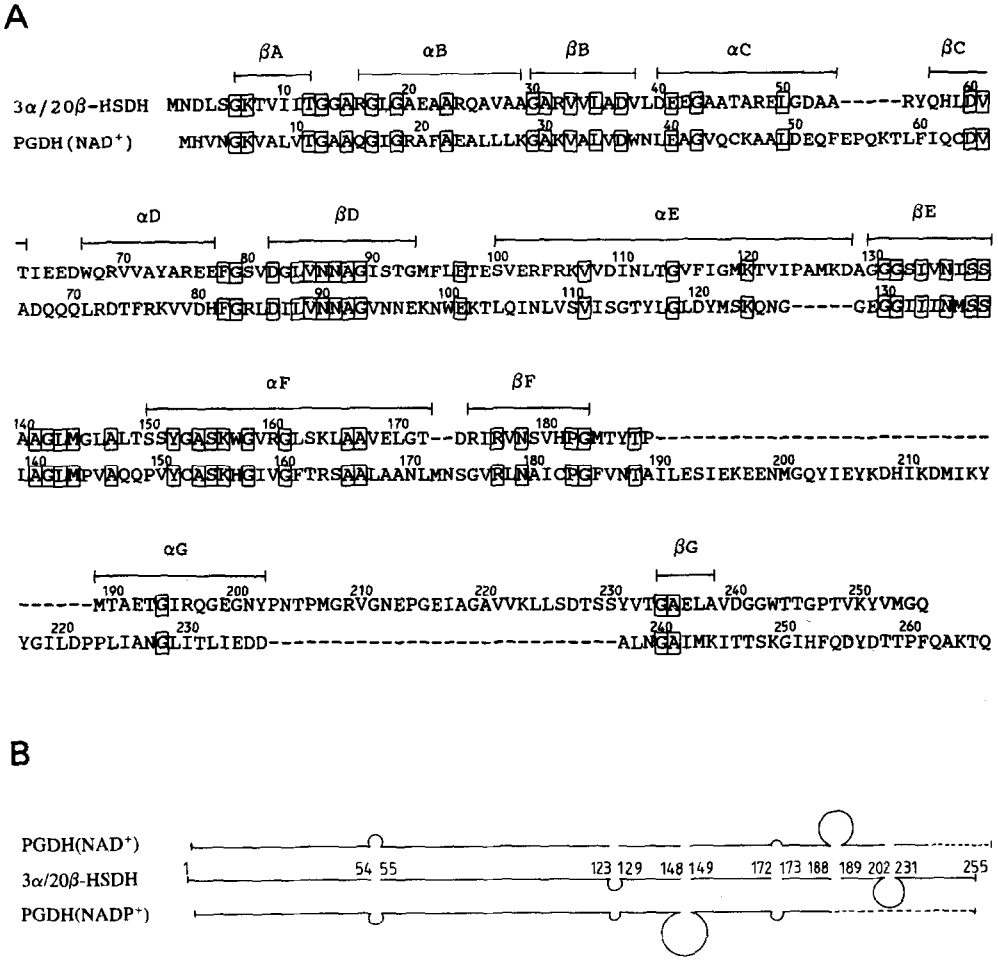

Fig. 1. Alignment (A) of NAD^+-dependent prostaglandin dehydrogenase with 3α/20β-hydroxysteroid dehydrogenase, and schematic representation (B) of the two prostaglandin dehydrogenases and the steroid dehydrogenase. In A, residue identities are boxed. Elements of secondary structure crystallographically determined in the steroid dehydrogenase [7] are shown above the sequence line. Positional numbers of each enzyme are given. Up to residue 189 of the prostaglandin dehydrogenase, alignments are considered final, the remaining parts more tentative. In the latter segments, supporting evidence for the alternative shown is given in the text. The choice of the position of αG gives a 33-residue extra loop in the prostaglandin dehydrogenase vs. the steroid dehydrogenase, as shown. In B, NAD^+-linked (top) and $NADP^+$-linked (bottom) dehydrogenases are given schematically relative to the 3α/20β-hydroxysteroid dehydrogenase (middle) determined crystallographically.

αG in the prostaglandin dehydrogenase (Fig. 1) because limited proteolysis of two different short-chain dehydrogenases results in cleavages in just this segment [16]. This suggests that the segment occupies a surface position on the enzyme molecule, as is commonly observed for inserted loops. Further, it makes the structure similar in this segment to that of another member of the short-chain dehydrogenase family, dihydropteridine reductase, which has also been crystallographically analyzed [8]. In the model, the αG helix would start at either His^{209} or Pro^{223}. Secondary structure predictions do not help to decide between these two alternatives since stretches of α-helices are predicted for both segments. We chose the latter alternative, aligning Met^{189}–Tyr^{202}, αG in 3α/20β-hydroxysteroid dehydrogenase, with Pro^{223}–Asp^{236} in the NAD^+-linked 15-hy-droxyprostaglandin dehydrogenase. We have not modelled the 33 residues between βF and αG (residues 190–222) or the last 17 residues of the protein chain (after position 248). The C-terminal part of the prostaglandin dehydrogenase is slightly longer than that of the steroid dehydrogenase and may contain an additional antiparallel β-strand similar to the one present in dihydropteridine dehydrogenase [8].

The alignment obtained has one long insertion toward the steroid dehydrogenase. Significantly, the $NADP^+$-linked prostaglandin dehydrogenase previously modelled also has a long insertion [1] (Fig. 1B). The two prostaglandin dehydrogenases modelled ([1] and this work), and the steroid dehydrogenase [7] and dihydropteridine reductase [8] crystallographically analyzed, establish a conserved, coenzyme-binding, N-terminal part, with parallel β-sheets and flanking α-helices, continuing into a highly variable C-terminal region that imparts distinct properties to individual members of the short-chain dehydrogenase family. The model of the NAD^+-linked prostaglandin dehydrogenase dimer is shown in Fig. 2. The residues making up the β-strands are more highly conserved with respect to the steroid dehydrogenase (50% identity, 81% conserved polarity) than other parts of the sequence. This is consistent with the β-sheet being internal, while α-helices and loops are on the surface and therefore more tolerant to variation.

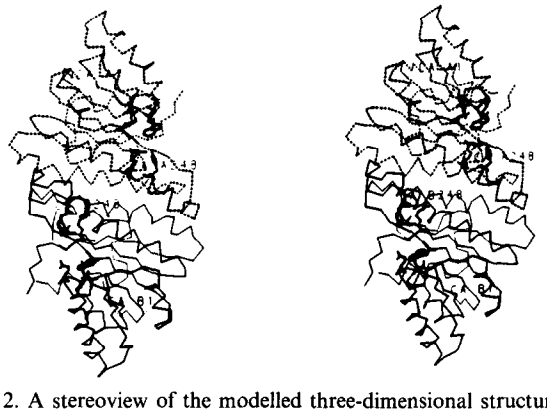

Fig. 2. A stereoview of the modelled three-dimensional structure of dimeric 15-hydroxyprostaglandin dehydrogenase. The trace of the α-carbon atoms are shown in solid lines for one subunit and dashed lines for the other. Amino acid side chains shown (bold) are at the active site; numbers 1 and 248 show the α-carbons of the first and last residues modelled. The view is nearly along the molecular two-fold axis relating the subunit association through the α-helices, αE and αF, at the Q axis interface. The unmodelled segment, residues 190–222, is not shown, explaining the loose ends (middle right in the dashed subunit).

Fig. 3. A close-up stereoview of the catalytic site in modelled 15-hydroxyprostaglandin dehydrogenase. The two subunits are drawn in solid and dashed lines as traces of their α-carbon atoms. Side chains shown (bold) are: Thr^{11}, Gln^{15}, Trp^{37}, Asn^{91}, Ser^{138}, Tyr^{151}, and Lys^{155}.

3.2. *Active site residues*

Many residues that are conserved in almost all short-chain dehydrogenases [6] are close to, or in the active site of, the steroid dehydrogenase [7], and are conserved also in the prostaglandin dehydrogenase model, i.e. Thr^{11}, Gly^{12}, Asn^{91}, Ala^{92}, Gly^{93}, Ser^{138}, Tyr^{151}, Lys^{155}, Pro^{183}, Gly^{184} and Asp^{36}.

Residues that interact with the coenzyme in the steroid dehydrogenase [7] and corresponding residues in the prostaglandin dehydrogenase model (within parentheses) are: Asp^{37} (Asp^{36}), Thr^{12} (Thr^{11}), Arg^{16} (Gln^{15}), Ile^{110} (Gly^{114}), Trp^{67} (Leu^{71}), Ile^{117} (Tyr^{121}), Asp^{109} (Ser^{113}) and Glu^{65} (Gln^{69}). Although only two of these residues are strictly conserved, all except one have conserved polarity. Other residues in the coenzyme-binding pocket are Trp^{37}, Val^{10}, and Met^{122}.

The inner part of the prostaglandin dehydrogenase substrate binding pocket is hydrophilic with many polar residues: Asn^{91}, Tyr^{151}, Lys^{155}, Ser^{138}, Gln^{15}, Asn^{95}, Gln^{148}, Glu^{97} and Asn^{187}. The outer part of the pocket is more hydrophobic, with Val^{145}, Pro^{183}, Phe^{185}, Leu^{139}, Met^{143}, Ala^{140} and Val^{186}. Positions of important residues at the active sites of the dimer are shown in Fig. 3.

3.3. *Subunit-subunit interactions*

The NAD^+-linked prostaglandin dehydrogenase is a

Fig. 4. A stereoview of the subunit interface of modelled 15-hydroxyprostaglandin dehydrogenase, viewed nearly parallel to the Q axis. The interface is comprised primarily of helices αE's (top layer) and αF's (partially visible; bottom layer), in a four-helix bundle. Side chains shown at the interfacial surface are Gln^{105} and Lys^{124} (from αE), and Gly^{157} and Phe^{161} (αF). Small residues (Gly and Ala) in αF permit the close packing of these two helices.

dimer, while the crystallographically analyzed 3α/20β-hydroxysteroid dehydrogenase is a tetramer with subunits related by a set of three mutually perpendicular rotation axes (Q, P and R). The most extensive contacts are those related by the Q axis and involve two helices, αE and αF. In our prostaglandin dehydrogenase model, these two helices have hydrophobic surfaces that could well contribute to the stabilization of dimer formation across the face as illustrated in Fig. 2. An alternative model for dimer formation would involve association about the P rotation axis which relates faces that contain the hydrophobic surfaces of αG but are otherwise less suited to dimer formation over their entire surface. The compatability of association of the hydrophobic side-chains of αE and αF is illustrated in Fig. 4. Specifically, internal hydrophobic interactions (Tyr^{116} with Tyr^{116}, Leu^{117} with Ile^{112}, Ala^{153} with Gly^{160}, Gly^{157} with Gly^{157}, and Phe^{161} with Pro^{149}, Trp^{100}) and polar interactions closer to the edges of the helices (Asp^{120}, Lys^{124} with Gln^{105}, and Ser^{164} with His^{156}) would stabilize the dimer formation about the Q axis. In this dimer, the active sites contain residues from only one of the two subunits.

3.4. *Conclusion*

The present modelling of NAD^+-linked prostaglandin dehydrogenase, together with the crystallographic analyses of 3α/20β-hydroxysteroid dehydrogenase [7] and dihydropteridine reductase [8], and the modelling of the $NADP^+$-linked dehydrogenase [1], establish common properties of the short-chain dehydrogenases:

(i) The structures, although exhibiting residue identities only at the 20% level, are clearly related in tertiary structure, and have single domain architectures and similar active site topologies.

(ii) Toward the C-terminal parts some organizational differences occur, with insertions or deletions of long segments as observed in both the crystallographically determined enzyme pair [7,8] and the two prostaglandin dehydrogenases (Fig. 1B).

(iii) In the oligomerization of these enzymes, major subunit interactions seem to be conserved, the face of the monomer containing the αE + αF helices apparently capable of forming stable subunit interactions in both the tetrameric and dimeric enzymes, highlighting the importance of the Q axis and suggesting that subunit interactions are possible to predict in this group of enzymes.

Acknowledgements: We are grateful to Dr. Zdzislaw Wawrzak for computational assistance. Support by the Swedish Medical Research Council (project 03X-3532), Karolinska Institutet, and The National Institutes of Health (DK26546) is gratefully acknowledged.

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